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Second-Generation Therapeutic DNA Lymphoma Vaccines

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14. ABSTRACT Lymphoma idiotype DNA vaccines have shown their therapeutic potential in preclinical studies; however, vaccine-induced anti-tumor effects need to be improved for translation of the vaccines into clinical use. Here we show that chemokine-fused idiotype DNA vaccines, when combined with cardiotoxin, which induced cellular infiltration at vaccination sites, were exceptional in their ability to provoke antitumor immunity in mice. The combined vaccination strategy significantly mounted both idiotype-specific T-cell responses and humoral immunity. Unexpectedly, vaccine-induced tumor protection was found to be intact in B-cell deficient mice, but was abrogated completely in T-cell-depleted mice. This is the first evidence showing that antibody response is not the immune mechanism for vaccine-induced tumor killing, supporting the significance of inducing T-cell immunity in designing idiotype vaccines. The potent immunostimulatory effect of myotoxins was immune-mediated, requiring recruitment of antigen-presenting cells for memory T-cell responses. These preclinical data highlight the translational potential of this novel idiotype DNA vaccine therapy against lymphoma.					
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Table of Contents

	<u>Page</u>
Introduction.....	4
Body.....	4
Key Research Accomplishments.....	6
Reportable Outcomes.....	6
Conclusion.....	6
Appendices.....	8

Annual Report

1. Introduction

Non-Hodgkin lymphomas (NHL) are a diverse group of lymphoproliferative neoplasms that differ in terms of their morphology, natural history, response to therapy, and prognosis. In the United States, NHL is the seventh most commonly diagnosed cancer and the sixth leading cause of death due to cancer. Although NHL is highly responsive to chemotherapy, the majority of patients relapse and eventually die of their disease. Therefore, novel, streamlined, potent therapeutic approaches to eliminate minimal residual disease are required to curb mortality from the disease. Therapeutic vaccination, in which a patient's immune system is "educated" to recognize and eliminate malignant cells, is a promising approach for eradication of minimal residual disease. The unique tumor immunoglobulin molecule from B-cell lymphoma, termed idiotype (Id), is an ideal tumor-specific antigen that can be used for vaccine generation. However, the development of an efficient vaccine formulation is largely limited by the inefficiency of vaccines in achieving potent and long-lasting antitumor immunities. Here, we developed a novel idioytppe vaccination strategy by: (1) fusion of lymphoma idiotype antigen to ligands of chemokine receptors present on antigen-presenting cells and (2) generation of a local inflammation at vaccination sites. The novel vaccination strategy has allowed us to achieve potent and long-term antitumor effects in murine lymphoma models.

2. Results

(1). The combined idiotype vaccination strategy activated tumor-specific T-cell immunities, which served as the major tumor-killing machinery.

Purpose: Determine antigen-specific cellular immunity induced by the novel vaccination strategy and its role in vaccine-induced tumor protection.

Study design: T-cell-mediated immunity was assessed by examining the activation of idiotype-specific and tumor-reactive T cells. Groups of 3 Balb/c mice were vaccinated with cardiotoxin plus MCP3-sFv20 lymphoma idiotype DNA vaccine or the idiotype vaccine alone. A total of three rounds of vaccination were given at two-week intervals. Mice were sacrificed 10 days after the final vaccination, and the immunized splenocytes were isolated and in vitro activated for 5 days with bone marrow-derived dendritic cells pulsed with 5 µg/mL A20 sFv H-2K^d epitope peptide (A20₁₀₆₋₁₁₄). The stimulated splenocytes were then seeded in a 96-well ELISPOT plate at 2.5×10^5 cells/well either with the peptides or irradiated A20 tumor cells at a 5:1 T cell/stimulator ratio for 48 hours. IFN-γ-producing T cells were detected by using an IFN-γ ELISPOT kit (BD Biosciences) and analyzed on a CTL ImmunoSpot[®] Analyzer (Cellular Technology Ltd.). The Student *t* test was used for statistical analysis.

The role of T cell-mediated immunity in protecting mice from tumor challenge was evaluated by in vivo T-cell depletion. T-cell depletion was achieved by treating the vaccinated mice with intraperitoneal injection of 200 µg of anti-CD8 (clone 2.43) and/or anti-CD4 (clone GK1.5) monoclonal antibodies according to the schedule (Figure 2). Depletion of T cells was confirmed by examining the population of CD3⁺CD8⁺ and/or CD3⁺CD4⁺ T cells in peripheral blood samples at designated time points (see in vivo T-cell depletion schedule). The vaccinated mice, with or without T-cell depletion, were then challenged with 2×10^5 tumor cells by intraperitoneal injection. Tumor growth and survival status were closely monitored for 80 days. Data were statistically analyzed by the Kaplan-Meier method, with log-rank tests to calculate *P* values. Abrogation of tumor protection by T-cell deletion would support the role of T-cell-mediated immunity in the vaccination-induced antitumor effect.

Results: Immunologic studies in vaccinated mice revealed the induction of tumor-specific cellular responses (Figure 1A). For example, the mean number of idiotype peptide-specific T cells per 2.5×10^5 splenocytes was 40 ± 2.6 in cardiotoxin-combined mice, compared with 18 ± 3.8 in mice receiving vaccine alone ($P < 0.01$) and 33 ± 8 compared with 7 ± 0.9 for tumor-specific T cells, respectively ($P < 0.05$). Furthermore, depleting CD8⁺ T cells in vivo after vaccination plus cardiotoxin was clearly associated with reduced tumor protection, and depletion of both CD4⁺ and CD8⁺ T-cell subsets abrogated protection completely (Figure 1b), suggesting a requirement for effector T cells in vaccine-induced antitumor immunity. T-cell depletion was confirmed by the absence of CD8 and/or CD4 T cells in the peripheral blood (Figure 2).

(2). Idiotype-specific antibody immunity was not required for the vaccine-induced tumor protection.

Purpose: Determine antigen-specific humoral immunity induced by the novel vaccination strategy and its role in vaccine-induced tumor protection.

Study design: Antibody response was determined by measuring serum levels of anti-idiotype antibodies. For this purpose, we vaccinated groups of five mice with cardiotoxin plus MCP3-sFv20 lymphoma idiotype DNA vaccine or the idiotype vaccine alone. Post-vaccination sera collected 10 days after each round of vaccination were used to detect anti-idiotype antibodies with ELISA method, by using recombinant A20 idiotype protein (provided by Favril Biotech) as the plate-coating antigen. The correlation between antibody immunity and vaccination-induced tumor protection was further determined by using B-cell-deficient J_H mice (Balb/c background, Taconic Farms). The wild-type and B-cell-deficient mice were vaccinated three times with cardiotoxin plus MCP3-sFv20 lymphoma idiotype DNA vaccine followed by tumor challenge with A20 murine lymphoma cells. Abrogation of tumor protection in B-cell-deficient J_H mice would confirm the role of antibody immunity in the vaccine-induced anti-tumor effect.

Results: The combined idiotype vaccination therapy mounted the humoral immunity, as demonstrated by substantially increased serum titers of antigen-specific antibodies after combined DNA vaccination plus cardiotoxin (Figure 3A), and maintained at high levels even after tumor challenge (Figure 3B). Surprisingly, in contrast with effector T cells, B cells were not required for tumor protection, as DNA vaccine plus cardiotoxin protected both genetically B-cell-deficient J_H mice and wild-type mice equally from tumor challenge (Figure 4A). Moreover, more than 80% of tumor-free J_H mice survived from primary challenge were highly resistant to tumor re-challenge, which provides evidence that anti-idiotype antibodies did not contribute principally to the memory anti-tumor immunity (Figure 4B). Antibodies have generally been thought to be the primary cellular mechanism underlying the antitumor effects of vaccines against lymphoma idiotype; however, the exact role of antibodies in eradicating tumor cells has not been fully shown. With antibody-deficient J_H mice, for the first time, we have shown that the antibody response was not required for idiotype-induced antitumor effects, even though the combination of vaccines with cardiotoxin strikingly enhanced serum anti-idiotype antibody titers. In contrast, depletion of effector T cells abrogated tumor protection, suggesting a primary role for cellular immunity in the mechanism of vaccine-induced tumor rejection.

(3). Administration of cardiotoxin results in sterile inflammation and recruitment of antigen-presenting cells at vaccination sites

Purpose: Determine mechanisms by which the novel vaccination strategy enhances cancer vaccine-induced adaptive immunity.

Study design: To study the microenvironment at cardiotoxin-injected muscle tissues, we injected groups of 3 mice with 100 μ L of 10 μ M cardiotoxin in the quadriceps, specimens of which were then collected at various times thereafter (24 hrs, Day 3, Day 5, Day 7 and Day 10), and fixed for hematoxylin and eosin staining. Further identification of the infiltrated immune cells was achieved by immunohistochemistry studies by using antibodies against cell markers for neutrophils (Gr-1), dendritic cells (CD11c), monocytes or macrophages (F4/80), NK cells (CD49b), B cells (B220), and T cells (CD3).

Results: Intramuscular injection of cardiotoxin caused notable cellular infiltration, which peaked after 3 to 5 days (Figure 5), coincident with DNA vaccine administration. Lymphocytes were not predominant (Figure 6 B220 and CD3). Rather, features of sterile inflammation were observed, characterized by initial infiltration of granulocytes within 24 hours, followed by monocytes/macrophages on Day 3 (Figure 6 Gr-1 and F4/80, respectively). Surprisingly, infiltrating dendritic cells (DC) were also observed by Day 3 (Figure 3a, CD11c). Given that both monocytes and dendritic cells are potent antigen-presenting cells required for the development of adaptive immunity, we thus hypothesized that **a localized inflammatory microenvironment would favor the development of an antitumor immunity by cancer vaccines.**

3. Key Research Accomplishments

(1). The novel vaccine therapy significantly potentiated vaccine-triggered and idiotypic-specific adaptive immune responses.

(2). Using B-cell-deficient mice, for the first time we have shown that antibody response were not required for idiotypic vaccine-induced antitumor effects. In contrast, by T-cell depletion we confirmed that cellular immunity is the principal immune mechanism for this novel vaccine therapy. This provides the rationale to include strategies of targeting T-cell immunity in designing the next generation of idiotypic vaccine.

(3). We identified that cardiotoxin induced sterile inflammation and recruitment of antigen-presenting cells at vaccination sites, which may play a critical role in triggering potent adaptive immunity.

4. Conclusion

In the previous year we developed a novel combined idiotypic DNA vaccine therapy which demonstrated its effectiveness in inducing potent anti-tumor effects on A20 murine lymphoma model. This year we further investigated the immune mechanism by which the novel vaccine therapy protects mice from tumor challenge. We found that both idiotypic-specific T-cell and antibody responses were potently induced by the vaccine therapy; however T-cell-mediated immunity is the major immune mechanism for eradicating tumor cells. Moreover, we found that cardiotoxin induced a sterile inflammation at vaccination sites, which results in recruitment of antigen-presenting cells. Thus, the immune microenvironment generated by local inflammation is a potential link between innate and adaptive immunity.

5. Future Plan

Given the finding of local infiltration of antigen-presenting cells at vaccination sites, we hypothesize that the inflammation microenvironment contains critical components in triggering innate immunity and may subsequently link innate to adaptive immunity. Our future studies will

then be focused on characterizing the immune microenvironment induced by cardiotoxin and identifying the mechanism by which this novel vaccine therapy potentiates antitumor immunity.

Figure 1

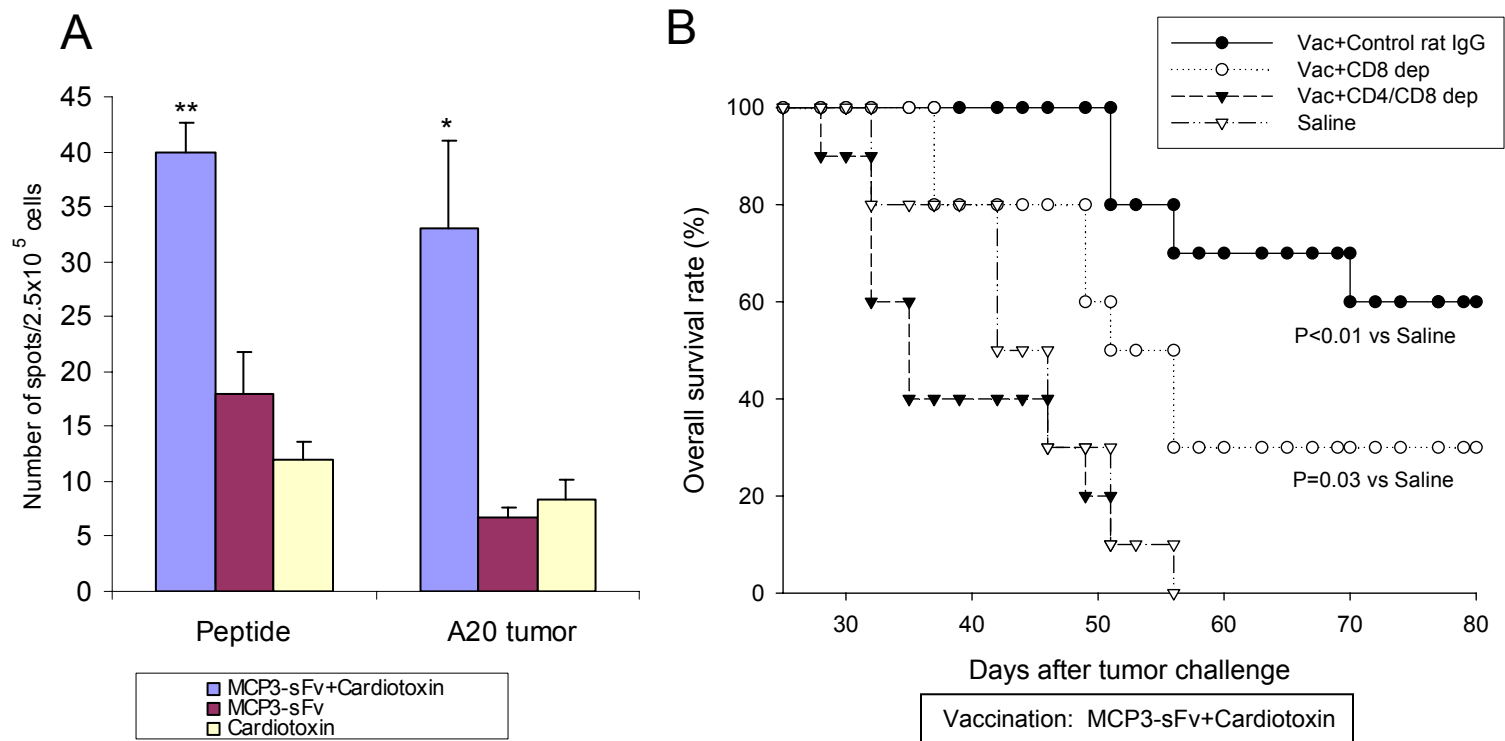
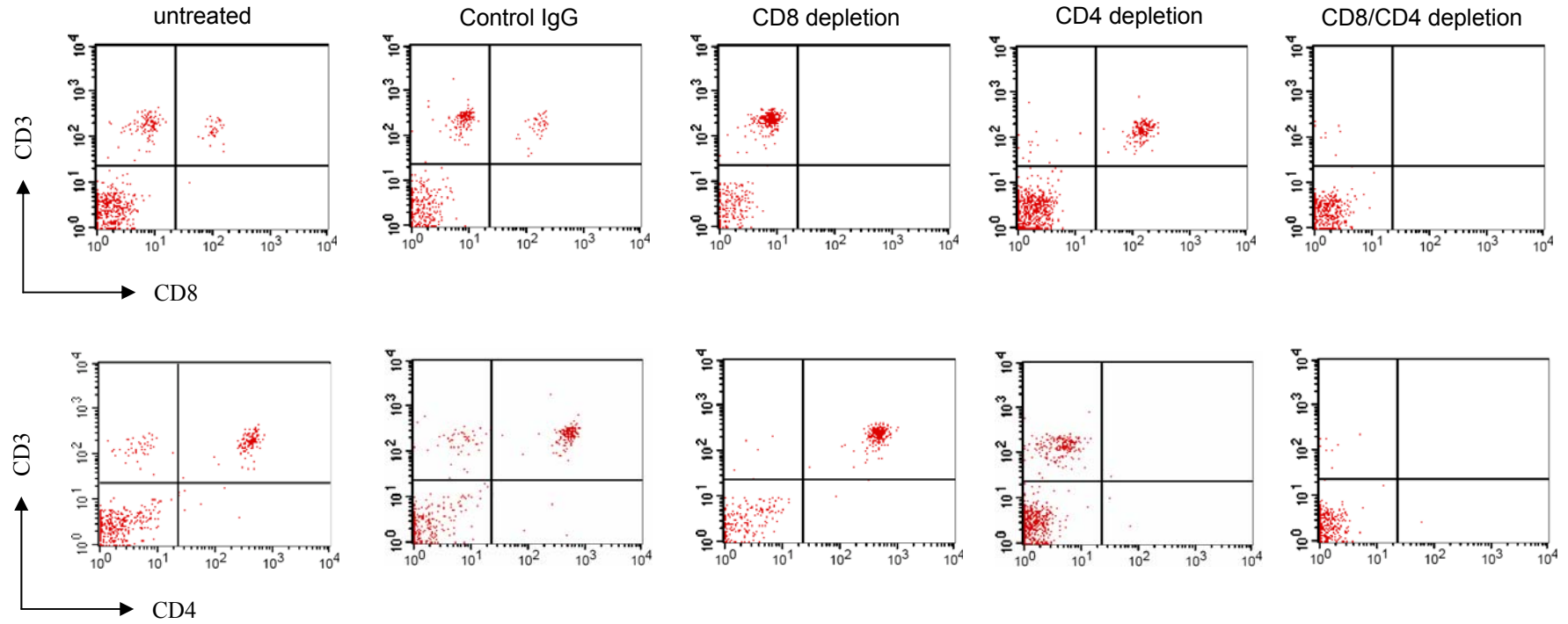


Figure 2



In vivo T cell depletion schedule

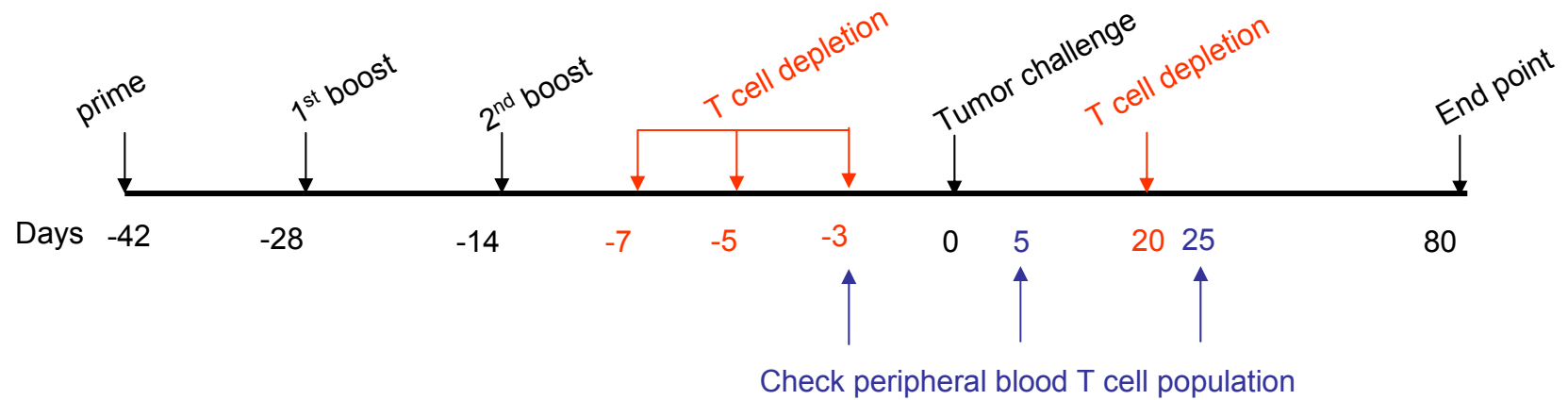


Figure 3

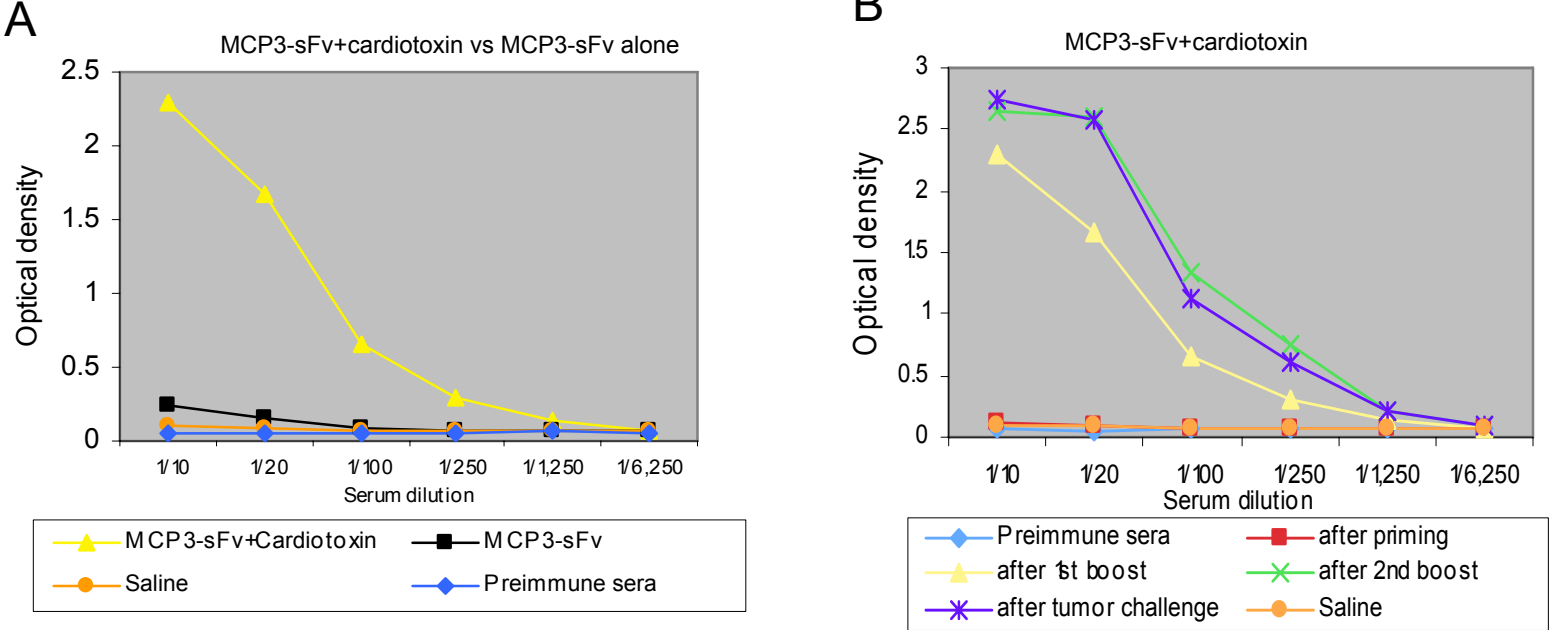


Figure 4

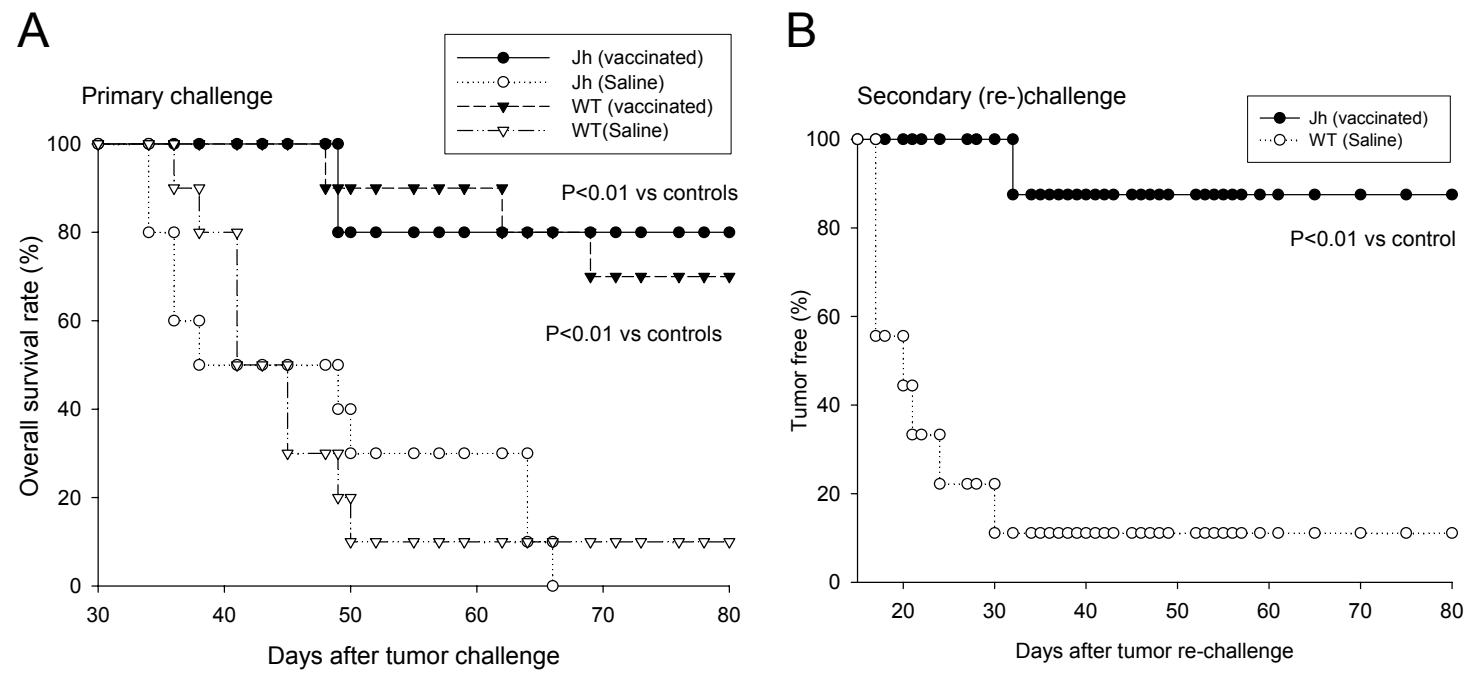


Figure 5

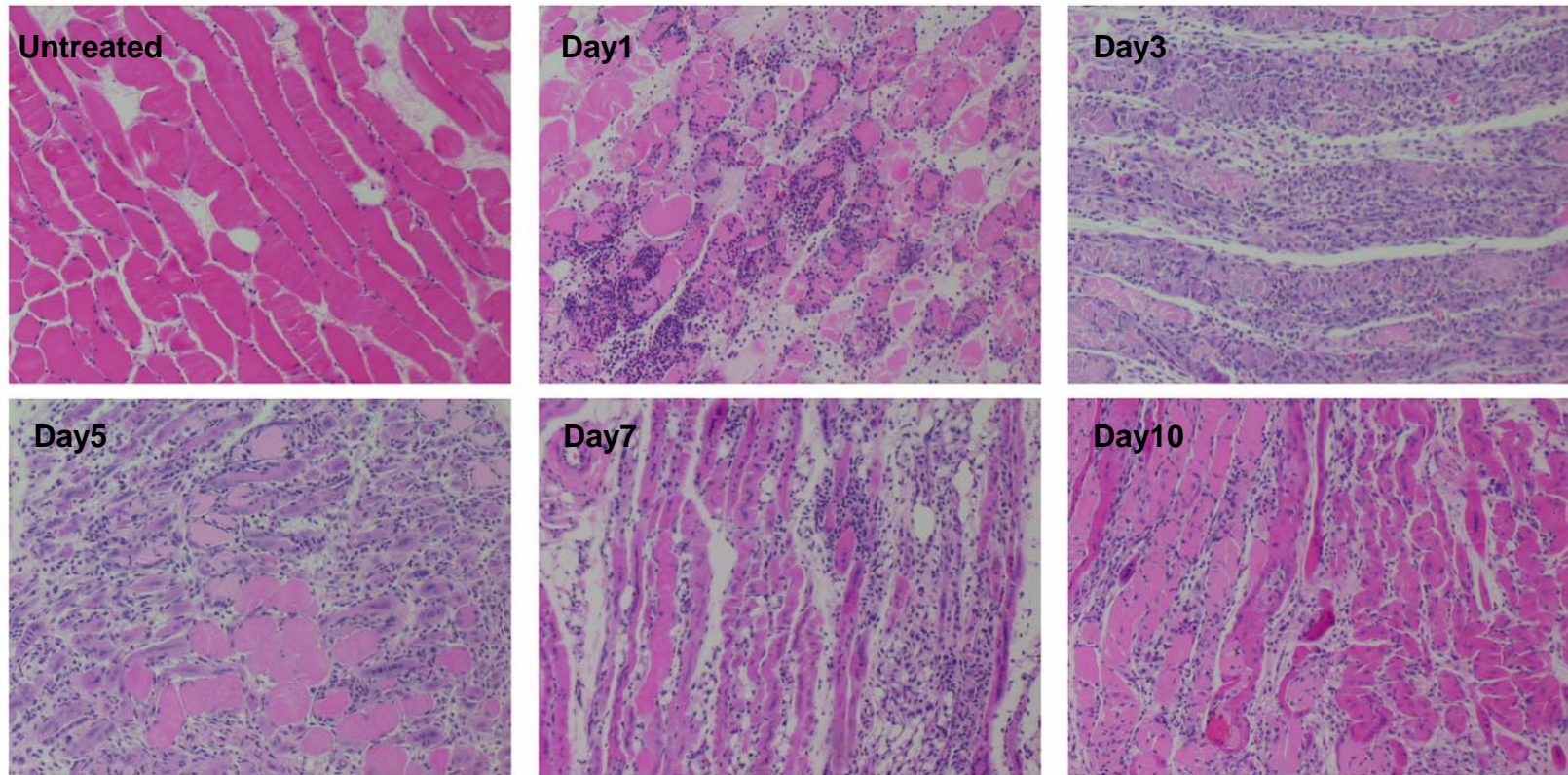


Figure 6

